Target Region Amplification Polymorphism (TRAP) analysis of the genus *Pelargonium*

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ABSTRACT

The current *Pelargonium* collection at the Ornamental Plant Germplasm Center (OPGC) consists primarily of cultivars originating from *P. ×hortorum* and *P. ×domesticum*. Since *Pelargonium* cultivars require vegetative propagation, the germplasm could be more efficiently maintained at OPGC if only the most genetically dissimilar accessions are retained; that necessitates a large scale molecular screening to identify redundant cultivars. TRAP (Target Region Amplification Polymorphism) has the advantage of producing a large number of markers through the use of sequence information that is already available. Our goal was to determine the feasibility of TRAP for analysis of this large collection. For most accessions, one or two primer combinations generated enough fragments to discriminate each accession, and similar clades were produced by cluster analysis of fragments amplified by different primer combinations. All the scorable fragments were polymorphic, 148 and 160 markers were scored for the two primer combinations. In another analysis, two multiplexed reactions yielded two dendrograms. In a dendrogram of plants with known species or pedigree, we observed that the accessions were generally in clades with others of the same species, but that the clades were less distinct when *P. ×hortorum* and *P. ×peltatum* hybrids were included. In spite of this, the clades were still divided according to their taxonomic sections. A second dendrogram was generated with the data from accessions that were potential duplicates, and most potential duplicates clustered close together. These results demonstrate that TRAP is an effective method for molecular characterization of ornamental collections.

INTRODUCTION

The US floricultural industry ranked *Pelargonium* as one of the three most important floral species for germplasm conservation (Tay, 2003)

In 2003 the OPGC (Ornamental Plant Germplasm Center, Columbus, Ohio) *Pelargonium* collection included approximately 900 accessions representing approximately 60 species.

Since *Pelargonium* cultivars require vegetative propagation, the germplasm could be more efficiently maintained at OPGC if only the most genetically dissimilar accessions were retained.

That necessitated a large scale molecular screening of the current collection to identify redundant cultivars and provide space for additional accessions representing the more than 200 remaining species.

Target Region Amplification Polymorphism (TRAP) was selected as an ideal method for analyzing a genebank's collection, because TRAP allows for evaluation of genetic variation that emphasizes specific traits of interest.

We intend to determine the genetic similarity of the accessions through molecular characterization by TRAP markers (Hu and Vick, 2003).

The advantages of this technique include:

- There is no need for extensive pre-PCR treatment of the DNA samples
- Many fragments can be amplified in a single PCR reaction
- The potential use of previously reported genetic information as the targeted primer

Objective and Hypothesis

Since TRAP had not yet been tested on Pelargonium species, our objective was to demonstrate that it could provide markers that distinguish between Pelargonium species.

We expected the accessions to segregate according to the known species designations, indicating that our long term goal of applying TRAP to evaluate the similarity of all the accessions in OPGC's collection should do the same.

Target Region Amplification Polymorphism

TRAP is a technique that combines the AT- and GC-rich primers of SRAP (Sequence-Related Amplification Polymorphism; Li and Quiros, 2001) with a third "fixed" primer that matches a gene of interest (Figure 1).

Each of the two arbitrary primers has a different fluorescent label, so the amplified DNA fragments can be detected by two different channels, each producing a separate image for one of the two fluorescently labeled primers on the same gel.

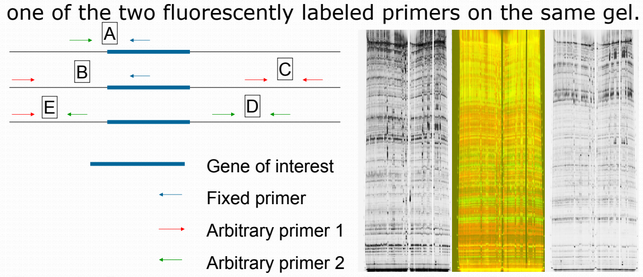

Figure 1: Fragment types observed in TRAP (Target Region Amplification Polymorphism) analysis include: those amplified between the fixed primer and one of the two arbitrary primers in the highest frequency (A and B above), and at a lower frequency those amplified between any combination of arbitrary primers (C, D and E above). Fragments amplified by the fixed primer alone would not be observed, because those are not labeled with fluorescent dyes. (Hu, 2006). Fragments observed with the fluorescent dyes are viewed in black and white one dye per image. The image above includes a color representation of the multiplexed reaction.

MATERIALS AND METHODS

Set 1: 46 Pelargonium accessions (9 species and 22 cultivars)

Fixed primers: QHA21B09a (*A. thaliana:* At5g65840.1)

QHF6H21L (*A. thaliana* BEL1-like homeobox 1)

Arbitrary primers: Sa12-700, Ga5-800

Set 2a: 74 accessions with 33 sets of potential duplicates

Set 2b: 182 accessions with known pedigrees/species

Fixed primer 1: TeloRA (targets the telomere region)

Arbitrary primers 1: Sa12-700, Ga5-800

Fixed primer 2: QHF6H12L (*A. thaliana* BEL1-like homeobox 1)

Arbitrary primers 2: Trap03-700, Trap13-800

TRAP: 50ng DNA, 1.5µL Qiagen 10X buffer, 1.5µL 25mM MgCl2, 1µL 5mM dNTPs, 0.3pmol of each fluorescently labeled arbitrary primer, 1pmol fixed primer, and 1.5 units of Taq polymerase.

94°C:2min, 5X(94°C:45sec, 35°C:45sec, 72°C:1min), 35X(94°C:45sec, 50°C:45sec, 72°C:1min), 72°C:7min.

RESULTS

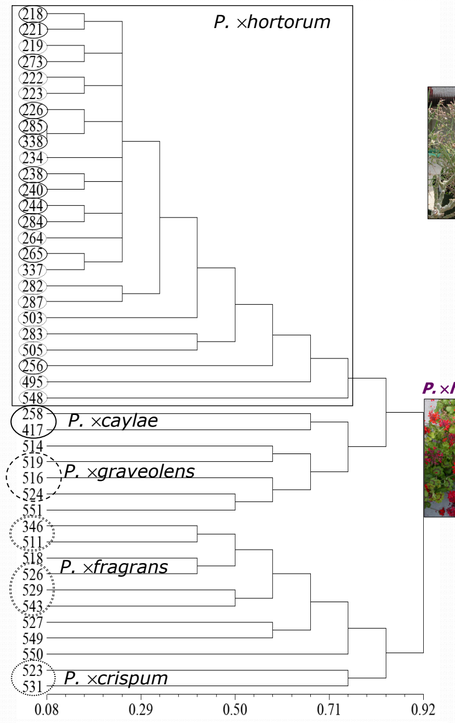

Figure 2: Dendrogram from set 1 (QHF6H21L). *P. ×hortorum* accessions are encased in a box on the dendrogram. Within that box, dotted circles indicate diploids and solid circles indicate tetraploids. Species numbers are circled on the dendrogram. Cophenetic correlation: r =-0.91163

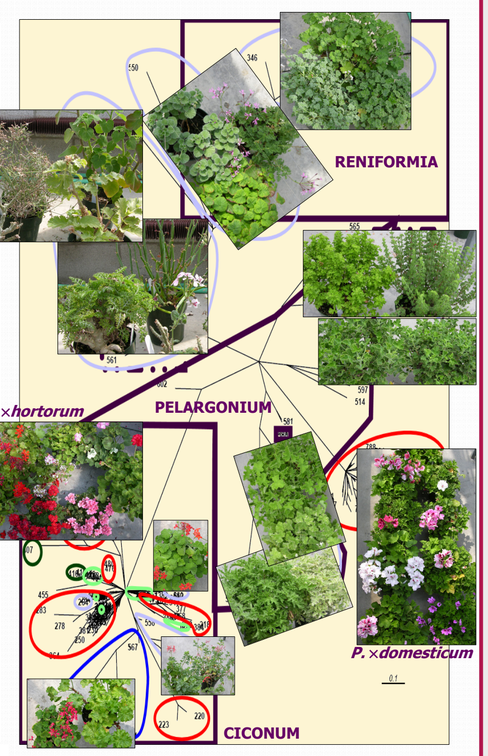

Figure 3: Dendrogram from set 2b with example pictures superimposed. Dark purple lines outline sections that are well represented. Section Ciconum includes *P. ×hortorum* (red), *P. ×peltatum* (green), and *P. ×zonale* (blue) hybrids, but *P. ×domesticum* hybrids clustered separately.

Set 2a	29 of 33 potential duplicates clustered 16 sets directly paired at least two	Other 4 were different species With the same cultivar name
Set 2b	Clustered by section P. ×domesticum clustered separately	Most hybrid species resulted in jumbled species within sections

CONCLUSIONS AND DISCUSSION

Based on the clustering patterns from our experiments that are consistent with the known pedigrees or species, we conclude that TRAP markers can distinguish between *Pelargonium* species.

This conclusion was based on our analysis of multiple sets of plants, using Bayesian analysis for sets 2 a and b, and UPGAMA for set 1. Set 1 indicated at species with known pedigrees clustered together, and set 2a indicated that identical or nearly identical accessions can be accurately identified.

Importantly, set 2b demonstrated the potential for confusion when hybrid cultivars are included, and yet also demonstrated that the clusters remained separated into taxonomic sections. Our analysis of the entire collection will require special attention to the hybrids of Section Ciconum.

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